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(54) **PHOSPHORAMIDITE DERIVATIVES OF GEMCITABINE FOR USE IN THE TREATMENT OF CANCER**

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C07H 19/10 (2006.01)

(52) **U.S. Cl.**

CPC **C07H 19/10** (2013.01)

(58) **Field of Classification Search**

CPC **C07H 19/10**
See application file for complete search history.

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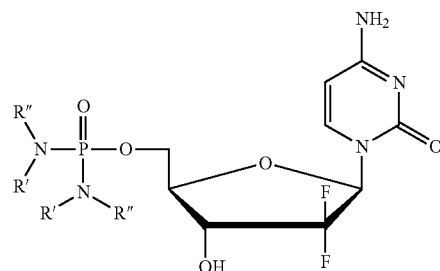
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(57) **ABSTRACT**

Phosphorodiamidate derivatives of e.g. gemcitabine are provided for use in treating cancer. In one embodiment, the two amidate motifs each comprises NR'R" where R' is H and R" is CR₅R₆CO₂R₇, where R₆ is H, R₅ is the side chain, including H and C₁, of a naturally occurring alpha amino acid, and R₇ is branched or unbranched, substituted or unsubstituted, acyclic or cyclic alkyl, including t-butyl-CH₂-, benzyl and C₃ to C₆ cycloalkyl. Formula (I).



27 Claims, No Drawings

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PHOSPHORAMIDITE DERIVATIVES OF GEMCITABINE FOR USE IN THE TREATMENT OF CANCER

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a national phase entry under 35 U.S.C. §371 of PCT International Application No. PCT/GB2011/001446, filed Oct. 5, 2011, which claims the priority benefit of GB 1016855.7, filed Oct. 6, 2010.

The present invention relates to nucleotide derivatives and their use in the treatment of cancer.

The nucleoside analogue gemcitabine is well established as an anticancer agent. It functions as an inhibitor of DNA synthesis after activation to its 5'-phosphate form.

International patent application no. PCT/GB2004/003148 (publication no. WO 2005/012327 A) discloses the use of phosphoramidate derivatives of gemcitabine to treat cancer. The derivatives all have both an aromatic group linked through O to P and a single amidate group linking through N to P. Compared to gemcitabine, the derivatives may display an enhanced potency with respect to cancer and/or a reduced toxicity.

D M Vail et al, 2007 AACR Annual Meeting, Los Angeles, Calif. presented an efficacy and safety profile of a prodrug of the anti-proliferative nucleotide analog 9-(2-phosphonyl-methoxyethyl) guanine used for the treatment of lymphoid malignancies. Guanine is derived from purine. The compound tested comprised a nucleoside phosphonate, i.e. a compound with a P—C link to the nucleoside base.

C R Wagner et al Med. Res. Rev. 2000, 20 417-451 discloses phosphoramidate diesters of FUDR and their use as anti-cancer agents.

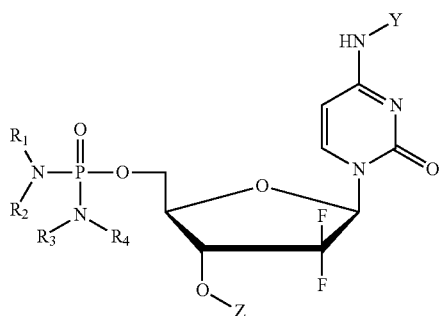
Each of D Cahard et al, Mini-Reviews in Medicinal Chemistry, 2004, 4, 371-382 and B C Jones et al, Antiviral Chemistry & Chemotherapy (1991) 2(1), 35-39 discloses phosphorodiamidates of AZT and their use as anti-viral agents.

There is a need to provide compounds in the treatment of cancer that show improved properties, particularly having regard to enhanced potency and/or reduced cytotoxicity.

It is an object of the present invention to provide such compounds.

It is a further object of the present invention to provide such compounds for use in the treatment of cancer.

According to the present invention there is provided a chemical compound according to formula (I):



wherein:

R_2 is selected from the group comprising $CR_5R_6CO_2R_7$ and C_1-C_6 alkyl and R_1 is selected from the group comprising H and C_1-C_6 alkyl,

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or R_1 and R_2 together with the N atom to which they are attached form a ring moiety comprising 5 to 8 ring atoms; R_4 is selected from the group comprising $CR_5R_6CO_2R_7$ and C_1-C_6 alkyl and R_3 is selected from the group comprising H and C_1-C_6 alkyl,

or R_3 and R_4 together with the N atom to which they are attached form a ring moiety comprising 5 to 8 ring atoms; where, independently for each of R_2 and R_4 :

R_5 and R_6 are selected, independently, from the group comprising the side chains of naturally occurring alpha amino acids; and

R_7 is selected from the group comprising C_1-C_{18} alkyl;

Y is selected from the group comprising H and $C(O)R$, where R is C_1-C_{18} alkyl;

Z is selected from the group comprising H and $C(O)R$, where R is C_1-C_{18} alkyl,

or a pharmaceutically acceptable derivative thereof.

Surprisingly, it has been found that phosphorodiamidate derivatives according to formula I are effective as potential chemotherapeutic agents in the treatment of cancer. Notably, compounds of formula (I) show enhanced anti-cancer potency and/or reduced cytotoxicity, compared to gemcitabine. The present invention thus provides the use of the compounds of the present invention in the treatment of cancer in *homo sapiens* and other mammals. In particular, compounds of the present invention are useful in the treatment of solid tumours, for example, in the treatment of colon cancer, breast cancer, pancreatic cancer, lung cancer and prostate cancer.

Although we do not wish to be bound by any theory, it is believed that in compounds of formula (I) it is the presence of the two amidate groups on the P atom, together with the identity of the groups R_1 , R_2 , R_3 , R_4 , R_5 , R_6 and R_7 , which contribute to the enhanced anti-cancer potency and/or the reduced cytotoxicity, compared to their nucleoside counterpart. Where R_2 and/or R_4 is the ester $-CR_5R_6CO_2R_7$, the anti-cancer potency exhibited varies with the choice of R_5 , R_6 and, especially, R_7 . Where R_2 and R_4 are each esters, the choice of each of R_1 and R_3 , and where R_2 and/or R_4 is not an ester, the choice of the remaining R_1 , R_2 , R_3 and R_4 groups on the respective amino N atoms also contribute to the anti-cancer potency. In one embodiment, each of R_2 and R_4 is $-CR_5R_6CO_2R_7$ and each of R_1 and R_3 is H, especially in such an embodiment where each of R_2 and R_4 is $-CH_5R_6CO_2R_7$ and each of R_1 and R_3 is H, R_2 and R_4 are the same.

It will be appreciated that when R_2 and/or R_4 is the ester group $-CR_5R_6CO_2R_7$, the respective moieties $-NR_1-$, $-CR_5R_6CO_2-$ and $-NR_3-CR_5R_6CO_2-$ of the phosphorodiamidate compounds of formula (I) correspond to the structure of that of alpha amino acids.

R_5 and R_6 are selected, independently, from the group comprising the side chains of naturally occurring alpha amino acids. By "naturally occurring alpha amino acids" is meant Alanine, Arginine, Asparagine, Aspartic Acid, Cysteine, Glycine, Glutamic Acid, Glutamine, Histidine, Hydroxylysine, Hydroxyproline, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine and Valine. The group comprising the side chains of these naturally occurring alpha amino acids, from which group R_5 and R_6 are independently selected, thus comprises the following moieties:

CH_3-	as present in Alanine
$H_2NC(=NH)NH[CH_2]_3-$	as present in Arginine
$NH_2C(O)CH_2-$	as present in Asparagine

-continued

HO ₂ CH ₂ —	as present in Aspartic Acid
HSCH ₂ —	as present in Cysteine
HO ₂ CH(NH ₂)CH ₂ SSCH ₂ —	as present in Cystine
H—	as present in Glycine
HO ₂ CH ₂ CH ₂ —	as present in Glutamic Acid
H ₂ N(O)CCH ₂ CH ₂ —	as present in Glutamine
C ₃ N ₂ HCH ₂ —	as present in Histidine
H ₂ NCH ₂ CH(OH)CH ₂ CH ₂ —	as present in Hydroxylysine
—CH ₂ CH(OH)CH ₂ —	as present in Hydroxyproline
CH ₃ CH ₂ CH(CH ₃)—	as present in Isoleucine
(CH ₃) ₂ CHCH ₂ —	as present in Leucine
H ₂ NCH ₂ (CH ₂) ₃ —	as present in Lysine
CH ₃ SCH ₂ CH ₂ —	as present in Methionine
PhCH ₂ —	as present in Phenylalanine
—CH ₂ CH ₂ CH ₂ —	as present in Proline
OHCH ₂ —	as present in Serine
CH ₃ CH(OH)—	as present in Threonine
C ₆ NH ₆ CH ₂ —	as present in Tryptophan
HOC ₆ H ₄ CH ₂ —	as present in Tyrosine
(CH ₃) ₂ CH—	as present in Valine.

The term “a side chain of a naturally occurring alpha amino acid” thus includes H and moieties comprising only one C atom, as well as saturated, unbranched C₃ moieties attached additionally at their terminal C atom to their respective amino N atom, in which instance, for R₂, R₁ is absent and for R₄, R₃ is absent.

By “a pharmaceutically acceptable derivative” is meant any pharmaceutically acceptable salt, ester, salt of such ester, hydrate, solvate, or crystalline form or metabolite or any other compound which upon administration to a recipient is capable of providing (directly or indirectly) a compound of formula (I).

In one embodiment, R₂ and R₄ are the same.

In one embodiment, R₂ and R₄ are each —CR₅R₆CO₂R₇.

Suitably, R₇ is selected from the group comprising branched and unbranched C₁-C₁₈ acyclic alkyl and C₃-C₈ cyclic alkyl, any of which acyclic and cyclic alkyl moieties may be substituted, with generally one, two or three substituents as set out below, and/or unsaturated. In one embodiment, R₇ is a branched, saturated and unsubstituted acyclic alkyl group, examples of which include i-propyl and t-butyl-CH₂—. In one embodiment, R₇ is an unbranched acyclic alkyl group, examples of which include methyl and ethyl. In one embodiment, R₇ is a substituted acyclic alkyl group, an example of which is benzyl. In one embodiment, R₇ is C₃-C₈ cycloalkyl, an example of which is cyclohexyl. Suitably, R₇ comprises only HC. In one embodiment, R₇ is selected from the group comprising methyl, ethyl, i-propyl, t-butyl-CH₂—, benzyl and C₃-C₆ cycloalkyl. R₇ is especially t-butyl-CH₂—. Where R₂ and R₄ are both esters, suitably R₇ is the same in each of R₂ and R₄ and each of R₁ and R₃ is H.

In one embodiment, where R₂ and/or R₄ are the ester, R₆ is H and R₅ is selected from the group comprising the side chains of naturally occurring alpha amino acids, especially from among said side chains comprising only H or HC. In such an embodiment where R₆ is H, R₅ is especially selected from the group comprising H, methyl, i-propyl, —CH₂Ph, —CH₂CH(CH₃)₂ and —CH(CH₃)(C₂H₅). In such an embodiment where R₆ is H, R₅ is more especially methyl and, even more especially where R₆ is H and R₅ is methyl, R₇ is t-Bu-CH₂—. Where R₂ and R₄ are both esters, suitably R₅, R₆ and R₇ are the same in each of R₃ and R₄ and each of R₁ and R₃ is H.

Where R₂ and/or R₄ is the ester and R₅ differs from R₆, the stereochemistry of the amino acid may be D or L. In one embodiment the amino acid has natural L stereochemistry at *CR₅R₆. In another embodiment the amino acid has D stereochemistry at *CR₅R₆.

In one embodiment, where R₂ and/or R₄ is the ester, each of R₅ and R₆ is methyl.

In one embodiment, each of R₁ and R₃ is the same. Especially, each of R₁ and R₃ is H. Where each of R₁ and R₃ is H, each of R₂ and R₄ is especially CR₅R₆CO₂R₇. More especially, where each of R₁ and R₃ is H and each of R₂ and R₄ is CR₅R₆CO₂R₇, R₆ is H and R₅ is particularly selected from the group comprising H, methyl, i-propyl, —CH₂Ph, —CH₂CH(CH₃)₂, —CH₂(i-C₃H₇) and —CH(CH₃)(C₂H₅), even more particularly R₅ is methyl. Even more especially, in any of such embodiments R₇ is selected from the group comprising methyl, ethyl, i-propyl, t-butyl-CH₂—, benzyl and C₃-C₆ cycloalkyl, and especially is t-Bu-CH₂—. Especially where each of R₁ and R₃ is H, each of R₂ and R₄ is the same ester.

Where R₁ and R₂ together with the N amino atom to which they are attached form a ring moiety comprising 5 to 8 ring atoms and/or R₃ and R₄ together with the N amino atom to which they are attached form a ring moiety comprising 5 to 8 ring atoms, the ring atoms can include additional hetero atoms such as O and/or further N and/or the ring atoms may be substituted or unsubstituted and/or the ring may be saturated or unsaturated. Suitably, in any one ring, the ring atoms can comprise up to a total of four hetero atoms. Suitably, where substituted, there will generally be one, two or three substituents present in any one ring. Substituents may any of: —CO₂R₇ (where R₇ is defined as set out above with respect to the moiety CR₅R₆CO₂R₇) and any of those moieties set out below as suitable substituents having regard to alkyl groups present in compounds of formula I. Where either or both of the combination of R₁ and R₂ and the combination of R₃ and R₄, together with the respective N atoms to which they are attached, form a saturated five membered ring moiety comprising four carbon atoms and have at least the substituent —CO₂R₇ on the appropriate C atom adjacent the N atom, the respective groups —NR₁R₂ and/or —NR₃R₄ may correspond to those defined above for R₁, R₂, R₃ and R₄, respectively, where one or both of R₅ and R₆ comprises the side chain of proline or hydroxyproline.

In one embodiment of the compound of formula I, R₁ and R₂ together with the N atom to which they are attached form a ring moiety comprising 5 to 8 ring atoms and R₃ and R₄ together with the N atom to which they are attached form a ring moiety comprising 5 to 8 ring atoms: in any such embodiment such respective ring moieties are suitably the same and are as set out above.

If the phosphate centre is chiral, it may be mixed R and S, or enriched in one stereoisomer.

In one embodiment, Y is H.

In one embodiment, Z is H.

In one embodiment, each of Y and Z is H, in which embodiment a compound according to formula I has a base derived from gemcitabine.

Any of the above specifically recited features may be combined together, as set out in claim 1, to provide a compound of formula I of the present invention. In particular, where X and Z is each H so that the nucleoside base is derived from gemcitabine, each of the above recited embodiments of the diamidate motif is particularly suitably combined with the gemcitabine derived base moiety.

Particularly preferred compounds of the present invention include:

- 2'-Deoxy-2',2'-difluoro-D-cytidine-5'-O-bis(ethoxy-L-alaninyl)-phosphate;
- 2'-Deoxy-2',2'-difluoro-D-cytidine-5'-O-bis(benzoyl-L-alaninyl)-phosphate;
- 2'-Deoxy-2',2'-difluoro-D-cytidine-5'-O-bis(cyclohexoxy-L-alaninyl)-phosphate;

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2'-Deoxy-2',2'-difluoro-D-cytidine-5'-O-bis(2,2-dimethyl-propoxy-L-alaninyl)-phosphate; and
2'-Deoxy-2',2'-difluoro-D-cytidine-5'-O-bis(iso-propoxy-L-alaninyl)-phosphate.

Reference in the present specification to an alkyl group means, unless otherwise stated, a branched or unbranched, cyclic or acyclic, saturated or unsaturated (e.g. alkenyl or alkynyl) hydrocarbyl radical. Where cyclic, the alkylene group is preferably C₃ to C₁₂, more preferably C₅ to C₁₀, more preferably C₅ to C₇. Where acyclic, the alkyl group is preferably C₁ to C₁₆, more preferably C₁ to C₆.

The alkyl groups may be substituted or unsubstituted. Where substituted, there will generally be one to three substituents present, preferably one substituent. Substituents may include halogen atoms, by which is meant F, Cl, Br and I atoms, and halomethyl groups such as CF₃ and CCl₃; oxygen containing groups such as oxo, hydroxy, carboxy, carboxyC₁₋₁₆alkyl, alkoxy, alkoyl, alkoyloxy, aryloxy, aryloyl and aryloxyloxy; nitrogen containing groups such as amino, C₁₋₆alkylamino, diC₁₋₆alkylamino, cyano, azide and nitro; sulphur containing groups such as thiol, C₁₋₆alkylthiol, sulphonyl and sulfoxide; heterocyclic groups which may themselves be substituted; alkyl groups as defined above, which may themselves be substituted; and aryl groups, by which is meant aromatic groups containing 5 to 14 ring atoms, for example phenyl or naphthyl, and heteroaromatic groups containing one, two, three or four, preferably one, heteroatoms selected, independently, from the group consisting of O, N and S, for example pyridyl, pyrrolyl, furanyl and thiophenyl, which aryl groups themselves may be substituted. Substituents on said heterocyclic, alkyl and aryl groups are as defined immediately above.

Reference in the present specification to alkoxy and aryloxy groups means, respectively, alkyl-O— (for example where alkyl is C₁ to C₁₆, preferably C₁ to C₆) and aryl-O— (for example where aryl is a 5 to 14 membered aromatic mono- or bifused ring moiety, optionally containing 1, 2, 3 or 4 heteroatoms selected, independently, from O, S and N, preferably aryl is phenyl).

Reference in the present specification to alkoyl and aryloyl groups means, respectively, alkyl-CO— (for example where alkyl is C₁ to C₁₆, preferably C₁ to C₆) and aryl-CO— (for example where aryl is a 5 to 14 membered aromatic mono or bifused ring moiety, optionally containing 1, 2, 3 or 4 heteroatoms selected, independently, from O, S and N, preferably aryl is phenyl).

Reference in the present specification to alkoyloxy and aryloxyloxy means, respectively, alkyl-CO—O— (for example where alkyl is C₁ to C₁₆, preferably C₁ to C₆) and aryl-CO—O— (for example where aryl is a 5 to 14 membered mono- or bifused aromatic ring system, optionally containing 1, 2, 3 or 4 heteroatoms selected, independently, from O, S and N, preferably aryl is phenyl).

Reference in the present specification to heterocyclic groups means groups containing one or more, pyrrolyl, imidazolyl, pyrazolyl, thiazolyl, isothiazolyl, oxazolyl, pyrrolidinyl, pyrrolinyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, tetrahydrofuranyl, pyranlyl, pyronlyl, pyridyl, pyrazinyl, pyridazinyl, piperidyl, piperazinyl, morpholinyl, thionaphthyl, benzofuranyl, isobenzofuryl, indolyl, oxyindolyl, isoindolyl, indazolyl, indolinyl, 7-azaindolyl, isoindazolyl, benzopyranyl, coumarinyl, isocoumarinyl, quinolyl, isoquinolyl, naphthridinyl, cinnolinyl, quinazolinyl, pyridopyridyl, benzoxazinyl, quinoxalinyl, chromenyl, chromanyl, isochromanlyl and carbolinyl.

According to a further aspect of the present invention there is provided a compound having formula I according to the

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present invention for use in a method of treatment, preferably in the prophylaxis or treatment of cancer, particularly in the prophylaxis or treatment of solid tumours, for example, of any one of colon cancer, breast cancer, pancreatic cancer, lung cancer and prostate cancer.

According to a further aspect of the present invention there is provided a method of prophylaxis or treatment of cancer, particularly a method of prophylaxis or treatment of solid tumours, for example, of any one of colon cancer, breast cancer, pancreatic cancer, lung cancer and prostate cancer, comprising administration to a patient in need of such treatment an effective dose of a compound having formula I according to the present invention.

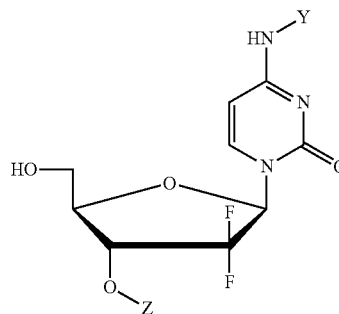
According to a further aspect of the present invention there is provided use of a compound having formula I of the present invention in the manufacture of a medicament for use in the treatment or prophylaxis of cancer, particularly a medicament for use in the treatment or prophylaxis of solid tumours, for example, of any one of colon cancer, breast cancer, pancreatic cancer, lung cancer and prostate cancer.

According to a further aspect of the present invention there is provided a pharmaceutical composition comprising a compound having formula I of the present invention in combination with a pharmaceutically acceptable excipient, carrier or diluent.

According to a further aspect of the present invention there is provided a method of preparing a pharmaceutical composition comprising the step of combining a compound having formula I of the present invention with a pharmaceutically acceptable excipient, carrier or diluent.

According to a further aspect of the present invention there is provided a process for preparing a compound of formula I, the process comprising reacting with phosphoryl chloride (POCl₃) a compound of formula II,

[III]



followed by reacting with amines of formulae R₁R₂NH and R₃R₄NH, wherein R₁, R₂, R₃, R₄, R₅, R₆, R₇, R, Z and Y have the meanings set out above.

The compound having formula I or pharmaceutical composition according to the present invention can be administered to a patient, which may be *homo sapiens* or animal, by any suitable means.

The medicaments employed in the present invention can be administered by oral or parenteral routes, including intravenous, intramuscular, intraperitoneal, subcutaneous, transdermal, airway (aerosol), rectal, vaginal and topical (including buccal and sublingual) administration.

For oral administration, the compounds of the invention will generally be provided in the form of tablets or capsules, as a powder or granules, or as an aqueous solution or suspension.

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Tablets for oral use may include the active ingredient mixed with pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavouring agents, colouring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while cornstarch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract.

Capsules for oral use include hard gelatin capsules in which the active ingredient is mixed with a solid diluent, and soft gelatin capsules wherein the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin or olive oil.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

For intramuscular, intraperitoneal, subcutaneous and intravenous use, the compounds of the invention will generally be provided in sterile aqueous solutions or suspensions, buffered to an appropriate pH and isotonicity. Suitable aqueous vehicles include Ringer's solution and isotonic sodium chloride. Aqueous suspensions according to the invention may include suspending agents such as cellulose derivatives, sodium alginate, polyvinyl-pyrrolidone and gum tragacanth, and a wetting agent such as lecithin. Suitable preservatives for aqueous suspensions include ethyl and n-propyl p-hydroxybenzoate.

The compounds of the invention may also be presented as liposome formulations.

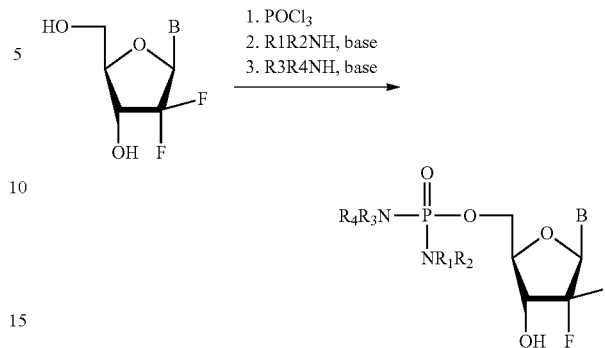
In general a suitable dose will be in the range of 0.1 to 300 mg per kilogram body weight of the recipient per day. A preferred lower dose is 0.5 mg per kilogram body weight of recipient per day, a more preferred lower dose is 6 mg per kilogram body weight of recipient per day, an even more preferred lower dose is 10 mg per kilogram body weight per recipient per day. A suitable dose is preferably in the range of 6 to 150 mg per kilogram body weight per day, and most preferably in the range of 15 to 100 mg per kilogram body weight per day. The desired dose is preferably presented as two, three, four, five or six or more sub-doses administered at appropriate intervals throughout the day. These sub-doses may be administered in unit dosage forms, for example, containing 10 to 1500 mg, preferably 20 to 1000 mg, and most preferably 50 to 700 mg of active ingredient per unit dosage form.

Embodiments of the present invention will now be described, by way of example only, with reference to the following examples, experimental procedures and experimental data.

Scheme 1 sets out a preparative pathway for preparing compounds of formula I. A person skilled in the art could use established synthetic methods to prepare close analogues related to the examples, by way of procedures disclosed in scheme 1.

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[Scheme 1]



wherein B is a nucleic acid base as set out in claim 1, such as cytosine, and R₁, R₂, R₃ and R₄ have the meanings set out in claim 1.

To ease synthetic access the free 3'-OH in the nucleoside may be pre-protected, for example with a BOC group, and then deprotected after phosphoramidate construction, using standard methods.

The structure and purity of the compounds can most easily be established by P-31 NMR, but C-13 or H-1 NMR are also useful, as is HPLC and MS.

The compounds can be assayed by many in vitro or in vivo methods, but in the first instance a cell proliferation assay is rapid and informative. Many tumour cell lines can be used for the assay, we herein report typical data for target compounds in HRT18 colon tumour cell line, using standard MTT assay methods, typical in many laboratories.

EXAMPLES

Preparation of Gemcitabine Diamidates

Standard Procedure A: Synthesis of Diamidates

To a stirring solution of 3'-Boc-gemcitabine (1.0 eq.) suspended in triethylphosphate (1.0 ml), cooled to 0° C. POCl₃ (2.0 eq.) was added dropwise under Ar atmosphere. The resulting reaction mixture was stirred 16 h at 0°-4° C. The crude mixture was diluted with anhydrous DCM (10 mL) and appropriate amino acid ester (5.0 eq.) was added, followed by dropwise addition of DIPEA (10.0 eq.) at -78° C. The reaction mixture was stirred for 96 h. The crude mixture was diluted with H₂O and extracted with DCM (6x20 mL). The organic phase were combined, dried under MgSO₄ and evaporated to yield a crude residue that was purified on silica gel using gradient of eluent (DCM/MeOH 99:1 to 97:3 to 95:5). (Excess of amino acid ester was removed by preparative TLC purification using DCM/MeOH 95/5 as an eluent).

Standard Procedure B: Synthesis of Diamidates

To a stirring solution of gemcitabine (1.0 eq.) suspended in triethylphosphate (1.0 ml), cooled to 0° C. POCl₃ (2.0 eq.) was added dropwise under Ar atmosphere. The resulting reaction mixture was stirred for 16 h at 0°-4° C. The crude mixture was diluted with anhydrous DCM (10 mL) and appropriate amino acid ester (5.0 eq.) was added, followed by dropwise addition of DIPEA (10.0 eq.) at -78° C. The reaction mixture

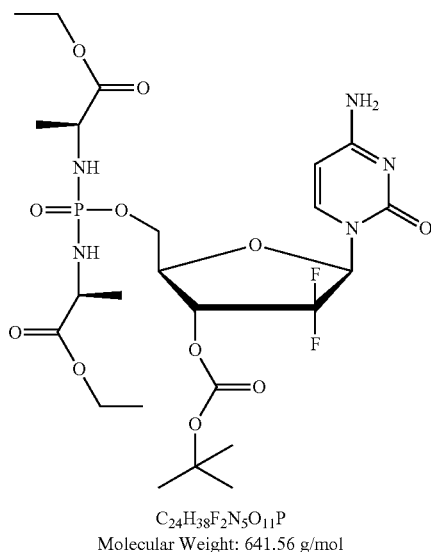
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was stirred for 96 h under Ar atmosphere at 0°-4° C. The crude mixture was diluted with H₂O and extracted with DCM (6×20 mL). The organic phase were combined, dried under MgSO₄ and evaporated to yield a crude residue that was purified on silica gel using gradient of eluent (DCM/MeOH 99:1 to 97:3 to 95:5). (Excess of amino acid ester was removed by preparative TLC purification using DCM/MeOH 95/5 as an eluent).

Standard Procedure C: Deprotection of Boc Group

A mixture of 3'-Boc-protected diamidate in TFA/DCM (1:1) was stirred at 0° C. for 2 hours. The solvents were evaporated and the residues was treated with saturated NaHCO₃, and extracted with EtOAc. The organic layers were combined, dried (MgSO₄), filtered, reduced to dryness and purified on silica gel with gradient of eluent (DCM/MeOH 95:5 to 92:8).

2'-Deoxy-2',2'-difluoro-3'-(tert-butoxycarbonyloxy)-D-cytidine-5'-O-bis(ethoxy-L-alaninyl) phosphate (1, MS151)



Prepared according to the standard procedure A from 3'-Boc-Gemcitabine (0.20 g, 0.55 mmol), POCl₃ (0.17 g, 0.17 mL, 1.10 mmol), and (Et)₃PO (1.0 mL), followed by addition of L-Ala ethyl ester HCl salt (0.42 g, 2.75 mmol), DIPEA (0.71 g, 0.95 mL, 5.55 mmol). Column purification gave the product as a white solid (0.075 g, 21%).

³¹P-NMR (MeOD, 202 MHz) δ 13.90

¹⁹F-NMR (MeOD, 121 MHz) δ -115.35 (d, J=251 Hz), -119.19 (d, broad signal, J=249 Hz)

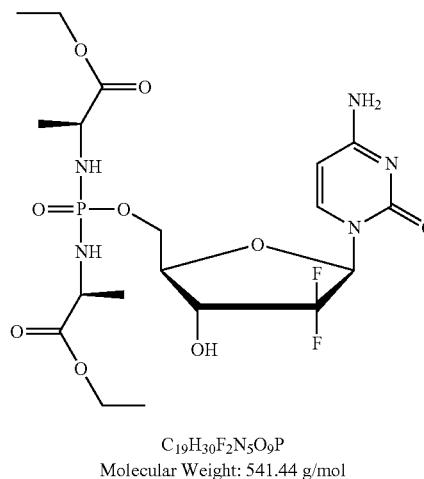
¹H-NMR (MeOD, 500 MHz) δ 7.71 (1H, d, J=8.16 Hz, H-base), 6.34 (1H, t, J=9.27 Hz, H-1'), 6.00 (1H, d, J=8.16 Hz, H-base), 5.27-5.22 (1H, m, H-3'), 4.37-4.33 (2H, m, 1×H-5', H-4'), 4.29-4.23 (1H, m, 1×H-5'), 4.22-4.16 (4H, m, 2×CH₂CH₃), 3.97-3.90 (2H, m, 2×CHCH₃), 1.52 (9H, s, C(CH₃)₃), 1.40 (6H, d, J=7.23 Hz, 2×CHCH₃), 1.28 (6H, apparent td, J=7.23 Hz, 2×CH₂CH₃)

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¹³C-NMR (MeOD, 125 MHz) δ 175.64 (d, ³J_{C-P}=5.70 Hz, C=O, ester), 167.76 (C=O, C-Ar, base), 157.60 (C-Ar, base), 153.18 (C=O, 3'-Boc), 143.02 (CH-Ar, base), 123.53 (apparent t, ¹J_{C-F}=260 Hz, CF₂), 96.86 (CH-Ar, base), 86.20-85.81 (m, broad signal, C-1'), 85.19 (C(CH₃)₃), 78.82 (d, ³J_{C-F}=6.50 Hz, C-4'), 74.16, 73.84 (2×d, ²J_{C-F}=18.0 Hz, 17.53 Hz, C-3'), 64.45 (d, ²J_{C-P}=3.91 Hz, C-5'), 62.37 (CH₂CH₃), 51.10 (d, ²J_{C-P}=3.90 Hz, CHCH₃), 27.87 (C(CH₃)₃), 20.86, 20.75 (2×d, ³J_{C-P}=5.43 Hz, CHCH₃), 14.53 (CH₂CH₃)

MS (ES+) m/e: 664 (MNa⁺, 100%), Accurate mass: C₂₄H₃₈F₂N₅O₁₁P required 641.56 found 642.24 (13%)

2'-Deoxy-2',2'-difluoro-D-cytidine-5'-O-bis(ethoxy-L-alaninyl)phosphate (2, CPF459)



Prepared according to the standard procedure C from the compound 1 (MS151) (0.075 g, 0.117 mmol), TFA/DCM (4 mL). Column purification followed by preparative TLC purification gave the product 2 as a white solid (7.0 mg, 11%).

³¹P-NMR (MeOD, 202 MHz) δ 13.91

¹⁹F-NMR (MeOD, 121 MHz) δ -118.39 (d, J=239 Hz), -119.83 (d, J=239 Hz)

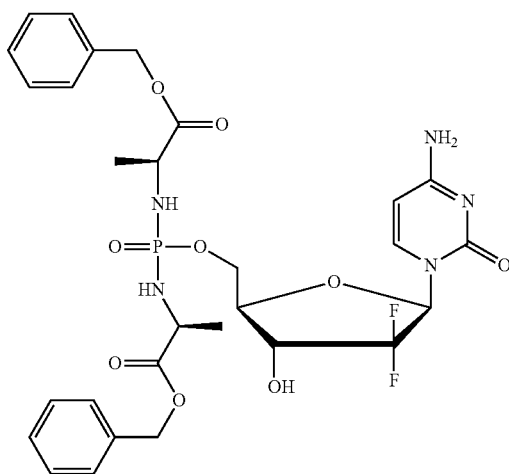
¹H-NMR (MeOD, 500 MHz) δ 7.70 (1H, d, J=7.41 Hz, H-base), 6.29 (1H, t, J=8.35 Hz, H-1'), 6.01 (1H, d, J=7.41 Hz, H-base), 4.36-4.22 (3H, m, 2×H-5', H-3'), 4.19-4.17 (4H, m, 2×CH₂CH₃), 4.07-4.05 (1H, m, H-4'), 3.96-3.89 (2H, m, 2×CHCH₃), 1.40 (6H, d, J=7.23 Hz, 2×CHCH₃), 1.30-1.27 (6H, m, 2×CH₂CH₃)

¹³C-NMR (MeOD, 125 MHz) δ 175.71 (d, ³J_{C-P}=4.15 Hz, C=O, ester), 167.70 (C=O, C-Ar, base), 157.79 (C-Ar, base), 142.67 (CH-Ar, base), 124.69 (apparent t, ¹J_{C-F}=259 Hz, CF₂), 96.77 (CH-Ar, base), 86.15-85.80 (m, broad signal, C-1'), 80.40 (broad signal, C-4'), 71.32, 71.13 (2×d, ²J_{C-F}=22.80 Hz, C-3'), 64.38 (d, ²J_{C-P}=5.41 Hz, C-5'), 62.34 (CH₂CH₃), 51.10 (d, ²J_{C-P}=3.90 Hz, CHCH₃), 20.85, 20.70 (2×d, ³J_{C-P}=5.79 Hz, CHCH₃), 14.47 (CH₂CH₃)

HPLC_b (H₂O/MeOH from 100/0 to 0/100 in 35 min) Rt 24.21 min

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2'-Deoxy-2',2'-difluoro-D-cytidine-5'-O-bis(benzyloxy-L-alaninyl)] phosphate (3, CPF457)



$C_{29}H_{34}F_2N_5O_9P$
Molecular Weight: 665.58 g/mol

Prepared according to the standard procedure B from Gemcitabine (0.25 g, 0.95 mmol), $POCl_3$ (0.29 g, 0.17 mL, 1.89 mmol), and $(Et)_3PO$ (1.0 mL), followed by addition of L-Ala benzyl ester Ts salt (1.67 g, 4.75 mmol), DIPEA (1.23 g, 1.65 mL, 9.50 mmol). Column purification followed by preparative TLC purification gave the product 3 as a white solid (0.079 g, 12%).

^{31}P -NMR (MeOD, 202 MHz) δ 13.87

^{19}F -NMR (MeOD, 121 MHz) δ -118.14 (d, $J=239$ Hz), -119.70 (d, broad signal, $J=239$ Hz)

1H -NMR (MeOD, 500 MHz) δ 7.67 (1H, d, $J=7.53$ Hz, H-base), 7.38-7.31 (10H, m, H-Ar), 6.27 (1H, t, $J=8.05$ Hz, H-1'), 5.97 (1H, d, $J=7.51$ Hz, H-base), 5.18-5.10 (4H, m, $2 \times CH_2Ph$), 4.31-4.17 (3H, m, H-5', H-3', H-5'), 4.02-3.95 (3H, m, H-4', $2 \times CHCH_3$), 1.38, 1.36 (6H, $2 \times d$, $J=7.14$ Hz, $2 \times CHCH_3$).

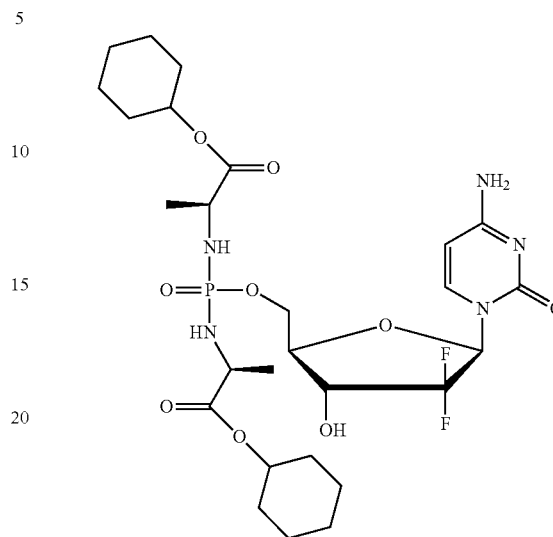
^{13}C -NMR (MeOD, 125 MHz) δ 175.43 (d, $^3J_{C-P}=5.40$ Hz, C=O, ester), 167.68 (C=O, C-Ar, base), 157.81 (C-Ar, base), 142.63 (CH-Ar, base), 137.29, 137.28 (C-Ar), 129.85, 129.66, 129.65, 129.40, 129.39, 129.32, 127.02 (CH-Ar), 123.69 (apparent t, $^1J_{C-F}=258$ Hz, CF_2), 96.87 (CH-Ar, base), 86.02 (apparent t, broad signal, $^2J_{C-F}=27.0$ Hz, C-1'), 80.46 (apparent t, $^3J_{C-F}=8.20$ Hz, C-4'), 71.28 (apparent t, $^2J_{C-F}=23.44$ Hz, C-3'), 68.00, 67.99 (CH_2Ph), 64.52 (d, $^2J_{C-F}=4.78$ Hz, C-5'), 51.15 (d, $^2J_{C-P}=5.41$ Hz, $CHCH_3$), 20.78, 20.67 ($2 \times d$, $^3J_{C-P}=5.53$ Hz, $CHCH_3$).

MS (ES+) m/e : 688.21 (MNa^+ , 100%), Accurate mass: $C_{29}H_{34}F_2N_5O_9P$ required 665.58 found 666.22 (3%)

HPLC_b ($H_2O/MeOH$ from 100/0 to 0/100 in 35 min) Rt 24.15 min

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2'-Deoxy-2',2'-difluoro-D-cytidine-5'-O-bis(cyclohexoxy-L-alaninyl)]-phosphate (4, CPF458)



$C_{27}H_{42}F_2N_5O_9P$
Molecular Weight: 649.62 g/mol

Prepared according to the standard procedure B from Gemcitabine (0.25 g, 0.95 mmol), $POCl_3$ (0.29 g, 0.17 mL, 1.89 mmol), and $(Et)_3PO$ (1.0 mL), followed by addition of L-Ala cyclohexyl ester HCl salt (0.99 g, 4.75 mmol), DIPEA (1.23 g, 1.65 mL, 9.50 mmol). Column purification followed by preparative TLC purification gave the product 4 as a white solid (0.029 g, 5%).

^{31}P -NMR (MeOD, 202 MHz) δ 13.94

^{19}F -NMR (MeOD, 121 MHz) δ -118.30 (d, $J=241$ Hz), -119.78 (d, broad signal, $J=245$ Hz)

1H -NMR (MeOD, 500 MHz) δ 7.70 (1H, d, $J=7.15$ Hz, H-base), 6.29 (1H, t, $J=7.97$ Hz, H-1'), 6.01 (1H, d, $J=7.51$ Hz, H-base), 4.79-4.73 (2H, m, $2 \times CH$ -cyclohexyl), 4.37-4.33 (1H, m, H-5'), 4.32-4.22 (2H, m, H-3', H-5'), 4.08-4.06 (1H, m, H-4'), 3.93-3.89 (2H, m, $2 \times CHCH_3$), 1.87-1.84 (4H, m, $2 \times CH_2$, cyclohexyl), 1.77-1.74 (4H, m, $2 \times CH_2$, cyclohexyl), 1.58-1.56 (2H, m, $2 \times CH$ of CH_2 -cyclohexyl), 1.48-1.33 (16H, m, 10H, CH_2 -cyclohexyl; 6H, $2 \times CHCH_3$)

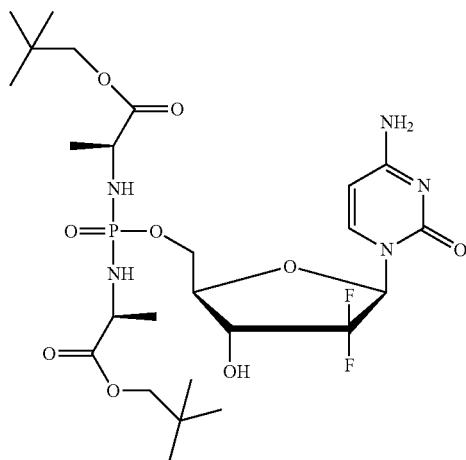
^{13}C -NMR (MeOD, 125 MHz) δ 175.64 (d, $^3J_{C-P}=5.37$ Hz, C=O, ester), 167.69 (C=O, C-Ar, base), 157.79 (C-Ar, base), 142.66 (CH-Ar, base), 123.66 (apparent t, $^1J_{C-F}=259$ Hz, CF_2), 96.85 (CH-Ar, base), 86.00 (apparent t, broad signal, $^2J_{C-F}=30$ Hz, C-1'), 80.48 (apparent t, $^3J_{C-F}=8.50$ Hz, C-4'), 74.66, 74.56 ($2 \times CH$ -cyclohexyl), 71.29 (apparent t, $^2J_{C-F}=25.0$ Hz, C-3'), 64.54 (d, $^2J_{C-P}=4.23$ Hz, C-5'), 51.13 (d, $^2J_{C-P}=13.0$ Hz, $CHCH_3$), 32.55, 32.53, 32.48, 32.34, 26.58, 26.44, 24.79, 24.69 (CH_2 -cyclohexyl), 21.15, 20.93 ($2 \times d$, $^3J_{C-P}=5.52$ Hz, $CHCH_3$)

MS (ES+) m/e : 672.26 (MNa^+ , 100%), Accurate mass: $C_{27}H_{42}F_2N_5O_9P$ required 649.62 found 650.28 (33%)

HPLC_b (H_2O/CH_3CN from 100/0 to 0/100 in 35 min) Rt 17.72 min

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2'-Deoxy-2',2'-difluoro-D-cytidine-5'-O-bis(2,2-dimethylpropoxy-L-alaninyl)]-phosphate (5, CPF460)



$C_{25}H_{42}F_2N_5O_9P$
Molecular Weight: 625.6 g/mol

Prepared according to the standard procedure B from Gemcitabine (0.25 g, 0.95 mmol), $POCl_3$ (0.29 g, 0.17 mL, 1.89 mmol), and $(Et)_3PO$ (1.0 mL), followed by addition of L-Ala neopentyl ester Ts salt (1.57 g, 4.75 mmol), DIPEA (1.23 g, 1.65 mL, 9.50 mmol). Column purification followed by preparative TLC purification gave the compound 5 as a white solid (0.041 g, 7%).

^{31}P -NMR (MeOD, 202 MHz) δ 13.93

^{19}F -NMR (MeOD, 121 MHz) δ -118.1 (d, $J=245$ Hz), -119.6 (d, $J=245$ Hz)

1H -NMR (MeOD, 500 MHz) δ 7.70 (1H, d, $J=7.58$ Hz, H-base), 6.29 (1H, t, $J=8.20$ Hz, H-1'), 6.01 (1H, d, $J=7.58$ Hz, H-base), 4.37-4.33 (1H, m, H-5'), 4.30-4.23 (2H, m, H-5', H-3'), 4.08-4.06 (1H, m, H-4'), 4.03-3.96 (2H, m, $2 \times CHCH_3$), 3.90, 3.93, 3.79, 3.73 (4H, $2 \times AB$, $J_{AB}=10.55$ Hz, $2 \times CH_2C(CH_3)_3$), 1.45, 1.43 (6H, $2 \times d$, $J=7.08$ Hz, $2 \times CHCH_3$), 0.97 (18H, s, $2 \times CH_2C(CH_3)_3$)

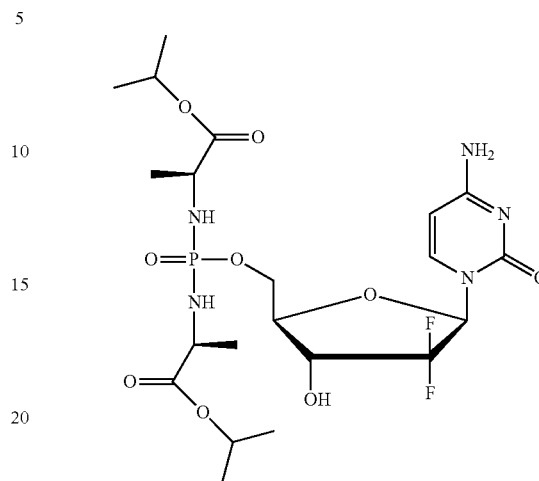
^{13}C -NMR (MeOD, 125 MHz) δ 175.71, 175.67 ($2 \times d$, $^3J_{C-P}=3.44$ Hz, C=O, ester), 167.70 (C=O, C—Ar, base), 157.79 (C—Ar, base), 142.68 (CH—Ar, base), 123.65 (apparent t, $^1J_{C-F}=258$ Hz, CF_2), 96.84 (CH—Ar, base), 86.04 (apparent t, $^2J_{C-F}=26$ Hz, C-1'), 80.48 (apparent t, $^3J_{C-F}=8.51$ Hz, C-4'), 75.46, 75.43 ($CH_2C(CH_3)_3$), 71.30 (t, $^2J_{C-F}=23.0$ Hz, C-3'), 64.57 (d, $^2J_{C-F}=4.73$ Hz, C-5'), 51.17 (d, $^2J_{C-P}=7.78$ Hz, $CHCH_3$), 26.7 ($CH_2C(CH_3)_3$), 21.08, 20.96 ($2 \times d$, $^3J_{C-P}=6.10$ Hz, $CHCH_3$)

MS (ES+) m/e : 648 (MNa^+ , 100%), Accurate mass: $C_{25}H_{42}F_2N_5O_9P$ required 625.60. found 626.28 (2%)

HPLC_b (H_2O/CH_3CN from 100/0 to 0/100 in 35 min) Rt 17.27 min

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2'-Deoxy-2',2'-difluoro-D-cytidine-5'-O-bis(iso-propoxy-L-alaninyl)]phosphate (6, CPF461)



$C_{21}H_{34}F_2N_5O_9P$
Molecular Weight: 569.49 g/mol

Prepared according to the standard procedure B from Gemcitabine (0.25 g, 0.95 mmol), $POCl_3$ (0.29 g, 0.17 mL, 1.89 mmol), and $(Et)_3PO$ (1.0 mL), followed by addition of L-Ala isopropyl ester HCl salt (0.80 g, 4.75 mmol), DIPEA (1.23 g, 1.65 mL, 9.50 mmol). Column purification followed by preparative TLC purification gave the product 6 as a white solid (0.037 g, 7%).

^{31}P -NMR (MeOD, 202 MHz) δ 13.97

^{19}F -NMR (MeOD, 121 MHz) δ -117.5 (d, $J=245$ Hz), -120.3 (d, $J=245$ Hz)

1H -NMR (MeOD, 500 MHz) δ 7.70 d, $J=7.20$ Hz, H-base), 6.29 (1H, t, $J=8.0$ Hz, H-1'), 6.00 (1H, d, $J=7.20$ Hz, H-base), 5.10-4.98 (2H, apparent septet, $J=6.40$ Hz, $2 \times H$, $CH(CH_3)_2$), 4.36-4.34 (1H, m, H-5'), 4.30-4.22 (2H, m, H-5', H-3'), 4.09-4.06 (1H, m, H-4'), 3.93-3.86 (2H, m, $2 \times H$, $CHCH_3$), 1.49, 1.47 (6H, $2 \times d$, $J=6.70$ Hz, $2 \times CHCH_3$), 1.29-1.26 (12H, m, $2 \times CH(CH_3)_2$)

^{13}C -NMR (MeOD, 125 MHz) δ 175.25 (d, $J_{C-P}=2.20$ Hz, C=O, ester), 167.70 (C=O, C—Ar, base), 157.81 (C—Ar, base), 142.68 (CH—Ar, base), 123.38 (apparent t, $^1J_{C-F}=257$ Hz, CF_2), 96.85 (CH—Ar, base), 85.94 (apparent t, $^2J_{C-F}=28.7$ Hz, C-1'), 80.46 (apparent t, $^3J_{C-F}=8.21$ Hz, C-4'), 71.28 (apparent t, $^2J_{C-F}=24.0$ Hz, C-3'), 70.12 (CH (CH_3)₂), 64.52 (d, $^2J_{C-P}=4.77$ Hz, C-5'), 51.17 (d, $^2J_{C-P}=8.6$ Hz, $CHCH_3$), 22.07, 22.05 (CH(CH_3)₂), 20.91, 20.82 ($2 \times d$, $^3J_{C-P}=6.3$ Hz, $CHCH_3$)

MS (ES+) m/e : 592.19 (MNa^+ , 100%), Accurate mass: $C_{21}H_{34}F_2N_5O_9P$ required 569.49. found 570.20 (10%)

HPLC_b (H_2O/CH_3CN from 100/0 to 0/100 in 35 min) Rt 12.15 min

Biological assay.

Human colon cancer cell line HRT18 were purchased from the European Collection of Animal Cell Cultures (ECACC, Salisbury, England). Cytotoxicity assay was based on MTT assay as we previously reported. The method is based on the ability of viable mitochondria to convert MTT, a soluble tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) into an insoluble formazan precipitate that is dissolved and quantified by spectrophotometry. A 96-well culture cell culture plate was used. Cells were

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counted with a haemocytomete counting chamber and a specific number (4000 per well) of cells were seeded to each well with culture medium (DMEM). Compounds, dissolved in DMSO, were series diluted (1:5) in culture medium, to cover a final concentration range between 0.128 and 2000 nM. The culture plate was incubated for 72 h at 37° C. The cells were washed twice with BSS. A solution of MTT in 0.5 mg/mL in culture medium was added into each well. The culture plate was then incubated at 37° C. for 4 h. MTT was then removed by aspiration. The crystals produced by MTT reagent within the cells were then extracted by the addition of 100 μ L of Triton X100 (10% in water). The cells were incubated at 4° C. for 24 h. The absorbance of the colorimetric products was then measured at a wavelength of 540 nm using a spectrophotometer (Titertecck).

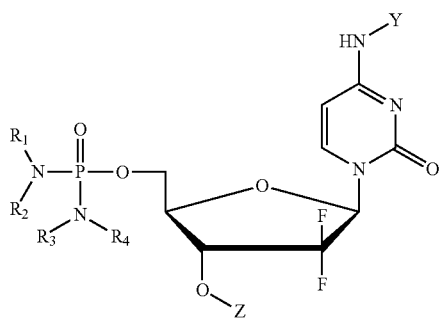
Data:

Cpd L-Ala Gemcitabine diamidate (R ₇)	EC50 (nM) Colorectal cell HRT18
CPF457	14
Bn	
CPF458	31
cycHex	
CPF459	42
Et	
CPF460	1.6
CH2tBu	
CPF461	3.2
iPr	
Gemcitabine	64

It can be observed that all of the diamidates are more potent than gemcitabine parent in this assay; some are up to 20 times more potent. Potency varies with the group R₇ of the ester as shown in the table, and also with the amino moiety (data not shown).

I claim:

1. A chemical compound having formula I:



wherein:

R₂ is selected from CR₅R₆CO₂R₇ and C₁-C₆ alkyl and R₁ is selected from H and C₁-C₆ alkyl, or R₁ and R₂ together with the N atom to which they are attached form a ring moiety consisting of 5 to 8 ring atoms;

R₄ is selected from CR₅R₆CO₂R₇ and C₁-C₆ alkyl and R₃ is selected from H and C₁-C₆ alkyl, or R₃ and R₄ together with the N atom to which they are attached form a ring moiety consisting of 5 to 8 ring atoms;

where, independently for each of R₂ and R₄;

R₅ and R₆ are selected, independently, from the side chains of naturally occurring alpha amino acids;

R₇ is selected from C₁-C₁₈ alkyl;

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Y is selected from H and C(O)R, where R is C₁-C₁₈ alkyl; and

Z is selected from H and C(O)R, where R is C₁-C₁₈ alkyl, or a pharmaceutically acceptable salt or crystalline form of a compound of formula I.

2. The compound according to claim 1, or a pharmaceutically acceptable salt or crystalline form of said compound, where R₂ and R₄ are the same.

3. The compound according to claim 1, or a pharmaceutically acceptable salt or crystalline form of said compound, where R₂ and R₄ are each CR₅R₆CO₂R₇.

4. The compound according to claim 1, or a pharmaceutically acceptable salt or crystalline form of said compound, wherein R₇ is selected from branched and unbranched, substituted or unsubstituted, and saturated or unsaturated C₁-C₁₈ acyclic alkyl and C₃-C₈ cyclic alkyl.

5. The compound according to claim 4, or a pharmaceutically acceptable salt or crystalline form of said compound, wherein R₇ is selected from methyl, ethyl, i-propyl, t-butyl-CH₂—, benzyl and C₃-C₆ cycloalkyl.

6. The compound according to claim 5, or a pharmaceutically acceptable salt or crystalline form of said compound, where R₇ is t-butyl-CH₂—.

7. The compound according to claim 1, or a pharmaceutically acceptable salt or crystalline form of said compound, wherein R₆ is H and R₅ is selected from the side chains of naturally occurring alpha amino acids.

8. The compound according to claim 7, or a pharmaceutically acceptable salt or crystalline form of said compound, wherein R₆ is H and R₅ is selected from H, methyl, i-propyl, —CH₂Ph, —CH₂CH(CH₃)₂ and —CH(CH₃)(C₂H₅).

9. The compound according to claim 8, or a pharmaceutically acceptable salt or crystalline form of said compound, wherein R₆ is H and R₅ is methyl.

10. The compound according to claim 9, or a pharmaceutically acceptable salt or crystalline form of said compound, wherein R₇ is selected from methyl, ethyl, i-propyl, t-butyl-CH₂—, benzyl and C₃-C₆ cycloalkyl.

11. The compound according to claim 10, or a pharmaceutically acceptable salt or crystalline form of said compound, wherein R₇ is t-butyl-CH₂—.

12. The compound according to claim 1, or a pharmaceutically acceptable salt or crystalline form of said compound, where the compound has natural L stereochemistry at *CR₅R₆.

13. The compound according to claim 1, or a pharmaceutically acceptable salt or crystalline form of said compound, wherein the compound has D stereochemistry at *CR₅R₆.

14. The compound according to claim 1, or a pharmaceutically acceptable salt or crystalline form of said compound, wherein each of R₅ and R₆ is methyl.

15. The compound according to claim 1, or a pharmaceutically acceptable salt or crystalline form of said compound, wherein each of R₁ and R₃ is the same.

16. The compound according to claim 15, or a pharmaceutically acceptable salt or crystalline form of said compound, wherein each of R₁ and R₃ is H.

17. The compound according to claim 16, or a pharmaceutically acceptable salt or crystalline form of said compound, wherein each of R₁ and R₃ is H and each of R₂ and R₄ is CR₅R₆CO₂R₇.

18. The compound according to claim 17, or a pharmaceutically acceptable salt or crystalline form of said compound, wherein each of R₁ and R₃ is H and each of R₂ and R₄ is CR₅R₆CO₂R₇ wherein R₆ is H and R₅ is selected from the side chains of naturally occurring alpha amino acids.

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19. The compound according to claim 18, or a pharmaceutically acceptable salt or crystalline form of said compound, wherein R_5 is selected from H, methyl, i-propyl, $-\text{CH}_2\text{Ph}$, $-\text{CH}_2\text{CH}(\text{CH}_3)_2$ and $-\text{CH}(\text{CH}_3)(\text{C}_2\text{H}_5)$.

20. The compound according to claim 19, or a pharmaceutically acceptable salt or crystalline form of said compound, wherein R_7 is selected from the group consisting of methyl, ethyl, i-propyl, t-butyl- CH_2- , benzyl and C_3 - C_6 cycloalkyl.

21. The compound according to claim 1, or a pharmaceutically acceptable salt or crystalline form of said compound, wherein R_1 and R_2 together with the N atom to which they are attached form a ring moiety consisting of 5 to 8 ring atoms and R_3 and R_4 together with the N atom to which they are attached form a ring moiety consisting of 5 to 8 ring atoms.

22. The compound according to claim 1, or a pharmaceutically acceptable salt or crystalline form of said compound, wherein each of Y and Z is H.

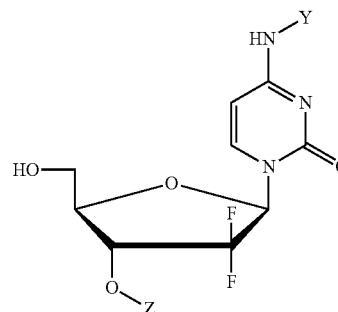
23. A method of treatment of cancer selected from colon cancer, breast cancer, pancreatic cancer, lung cancer and prostate cancer, comprising administering to a patient in need of such treatment an effective dose of a compound according to claim 1, or a pharmaceutically acceptable salt or crystalline form of said compound.

24. A pharmaceutical composition comprising a compound according to claim 1, or a pharmaceutically acceptable salt or crystalline form of said compound, in combination with a pharmaceutically acceptable carrier, diluent or excipient.

25. A method of preparing a pharmaceutical composition comprising the step of combining a compound according to claim 1, or a pharmaceutically acceptable salt or crystalline form of said compound, with a pharmaceutically acceptable excipient, carrier or diluent.

26. A process for preparing a compound of formula I according to claim 1, the process comprising reacting with phosphoryl chloride (POCl_3) a compound of formula (II):

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[III]

followed by reaction with $R_1R_2\text{NH}$ and $R_3R_4\text{NH}$ wherein

R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R, Y, and Z have the meanings described in claim 1, wherein when Z is H, the 3'-OH is pre-protected prior to reaction with the POCl_3 and deprotected after reaction with $R_1R_2\text{NH}$ and $R_3R_4\text{NH}$.

27. A compound selected from the group consisting of:

2'-deoxy-2',2'-difluoro-D-cytidine-5'O-bis(ethoxy-L-alaninyl)-phosphate;

2'-deoxy-2',2'-difluoro-D-cytidine-5'O-bis(benzyloxy-L-alaninyl)-phosphate;

2'-deoxy-2',2'-difluoro-D-cytidine-5'O-bis(cyclohexoxy-L-alaninyl)-phosphate;

2'-deoxy-2',2'-difluoro-D-cytidine-5'O-bis(2,2-dimethylpropoxy-L-alaninyl)-phosphate; and

2'-deoxy-2',2'-difluoro-D-cytidine-5'O-bis(isopropoxy-L-alaninyl)-phosphate, or a pharmaceutically acceptable salt or crystalline form of said compound.

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